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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 7 JULY, 1949 NO. 7

NUTRITIONAL DISEASES IN CHINA

Owing to the vastness of the country and the fact that only limited studies have been made, there are insufficient data to enable us to form an accurate knowledge of the incidence of deficiency diseases in China; however, it is safe to say that nutritional deficiency diseases are quite prevalent. From available data it may be stated that the incidence of various types of deficiency diseases differs from place to place due largely to variations in dietary habits. For instance, in the rice-eating regions in central and southern China the incidence of beriberi is high, while in the north where the people have wheat and other cereals as their staple diet, the incidence is low. Vitamin A deficiency is common throughout different parts of the country with a somewhat higher incidence in the north. The low fat content of the diet may be responsible for the poor utilization of the carotene which may be present in sufficient amount. Riboflavin deficiency is also common in places where such studies have been made. Rickets, osteomalacia, or vitamin D deficiency are more common in the north than in the south. This may be explained by the fact that people in the north, especially women and children, confine themselves most of the time indoors during the long winter months, while in southern China the people are exposed to sunshine during the comparatively mild winter.

Pellagra and scurvy are rather rare in the country except under conditions of great stress, when outbreaks were found, as among refugee groups or in famine areas. The Chinese diet in general is low in fat, vitamins, animal protein, and calcium. These insufficiencies in calcium and protein over generations may account in part for the small stature of the Chinese people. Anemia as a result of blood loss from various causes is common, but only a few cases are due primarily to iron deficiency in the diet. Simple goiter as a result of iodine deficiency is prevalent in certain areas far removed from the coast.

Vitamin B_1 deficiency. From description in the old Chinese literature, beriberi apparently was known in China over two thousand years ago. It was described as being prevalent among scholars from southern China during the years 307 to 312 A.D. Its prevalence among troops and inhabitants of besieged cities was recorded in the years 529 and 605 A.D. In a publication entitled "Thousand Golden Prescriptions" written in the seventh century, there is described a method of preparing rice polishings for the treatment of beriberi. Up to the present time beriberi has remained prevalent in southern China where the people prefer a highly polished rice. It is of interest that certain people in southern China like to eat raw fish which has been found to contain thiaminase, an enzyme which destroys vitamin B_1.

During the postwar period, with the increased supply of fresh food and of vitamin preparations, there was a considerable decrease in incidence of frank beriberi in urban areas. However, with the subsequent poor economic condition of the country and the difficulty of many to obtain sufficient food, latent beriberi again becomes prevalent.

Riboflavin deficiency. This was notoriously prevalent among war refugees in Shanghai in 1939 and 1940, particularly among children, where often as many as 45 to 50 per cent showed signs of riboflavin deficiency. The common signs of riboflavin deficiency most frequently seen are conjunctival congestion, cheilosis, and glossitis.

Seborrheic dermatitis as a sign of riboflavin deficiency is rarely seen.

Niacin deficiency. Prior to the war (up to 1937), pellagra among Chinese was comparatively rare. Only 49 cases were reported in the literature and this number includes 30 cases from a prison camp. In Shanghai in 1937, during the six months following the outbreak of hostility with the Japanese and within the subsequent period of one year, the writer observed and treated 86 cases. All of them showed dermatitis either symmetrically distributed or centrally placed, usually with a brown color and rough, wrinkled skin, adjoining abruptly the normal skin. Many of the patients gave a history of having had diarrhea for a number of days either before or during the development of the dermatitis. Dementia was observed in one of the 86 cases of this series.

During the last few years, even before the surrender of the Japanese, pellagra became rare again. From 1943 to 1948, a period of five years, less than 10 cases were seen in the city of greater Shanghai with a population of four and a half millions. Apparently the people were able to include in their diet some food rich in niacin or tryptophan.

Vitamin A deficiency. Two conditions, namely keratosis of the skin and xerophthalmia, are commonly observed. Night blindness is another common vitamin A deficiency sign, but few patients come to the hospital with such a complaint. A survey made among hospital staffs, nursing students, and hospital patients, as well as students of certain schools, by the dark adaptation tests, reveals a high incidence of mild night blindness, which responded readily to vitamin A therapy. Night blindness was recorded in the old Chinese medical literature as early as 610 A.D.

Measures to improve the Chinese diet. Under ordinary conditions, the diet of the Chinese is sufficient in quantity, but poor in quality. In time of war and famine there is also an insufficient quantity. By far the largest amount of a single food consumed by the population is rice. It forms a fairly satisfactory diet for the adult when it is eaten with green, leafy vegetables and some pork, since pork supplies the animal protein, fat, and certain vitamins which rice lacks. Pigs can be raised most economically and have been raised in China for centuries, but the quantity is not great enough and the price is too high to be generally used. This industry should be promoted and encouraged.

For centuries, soybeans and soybean products have been used in the Chinese diet. They play an important role in the nutrition of the people since they contain about 40 per cent of good protein, 20 per cent of fat, generous amounts of vitamin B-complex, and certain minerals. However, their use as food in the form of beans has not been extensive; instead, various soybean preparations are used and the increased cost has reduced the quantity consumed by the people. For instance, in the production of soybean milk about one fifth of the bean is lost in a residue which is not eaten by man. A more extensive cultivation of soybeans and an educational campaign on the nutritive value of soybeans would help toward better nutrition.

Soybeans are one of the main sources of vegetable oil in China. Unfortunately, the residue from the oil extraction, which is mostly good protein, is not utilized for human food, but is sold as fertilizer for the rice fields. This residue should form an important source of protein food for the mass of Chinese people.

China produces sixty-seven billion eggs per year, which number is estimated to be barely enough for the requirements of the Chinese people. However, millions of eggs are exported yearly. It will be necessary, therefore, to devise a policy to keep the eggs in the country or else to barter them for some cheaper foodstuff with a good nutritional value, such as skimmed milk powder.

Most Chinese like fish, but the fishing in-

dustry has not been developed on a large enough scale to bring the price down to a practical level. Improvement of the transportation system will be necessary to bring this valuable food to the interior.

A large-scale dairy industry is not practicable in China since in the thickly populated areas pasture land is not available and all cereals have to be consumed by human beings. In the few thinly populated areas such an industry might be inaugurated, but the transportation and refrigeration costs at the present would make the price of milk prohibitive.

The processes of parboiling and of enriching rice are two means which may be employed to ensure a rice with a high vitamin B_1 content. As soon as the economic conditions of the country are stabilized such processes should be developed.

Nutrition studies and plans for nutrition improvement. During the past thirty years nutrition studies in China were carried out mainly in a few universities and research institutes. Four years ago a Nutrition Institute was inaugurated in the National Institute of Health. It has undertaken a number of research problems in laboratories, clinics and hospitals, and in the field, producing certain educational pamphlets, models, posters, and other materials for the popularization of nutrition knowledge. Prior to this, popular nutrition education had been undertaken by the China Nutritional Aid Council which, from 1938 to 1945, had fed over a million undernourished children with a supplemented soybean milk, and also fostered much popular nutrition knowledge.

In January 1944, a plan of nutrition improvement was drawn up by the Nutrition Conference held in Chungking and submitted to the National Food Conference and various government organizations for adoption. Briefly it proposes: (1) An emergency program which includes: (a) increased production of certain selective foods, (b) improvement of cooking methods and food habits, and (c) specific recommendation to the diet of mothers, infants, children, students, and soldiers. (2) A long-range program of nutrition education including: (a) popularization of nutrition education through schools, mothers' clubs, etc., with lectures, movies, demonstrations, exhibits, slogans, etc., and (b) training of personnel such as nutrition specialists, nutrition workers, canteen workers, and cooks. (3) Nutrition research including: (a) establishment of a Central Nutrition Research Institute, and (b) establishment of a number of nutrition centers in different districts. Emphasis is placed on studies of local food values, dietary problems, surveys of nutritional status, and public health aspects of nutrition.

Despite the difficulties of wartime conditions, some progress has been made. More people in cities have become nutrition conscious and in a number of places nutritional deficiency diseases are fewer.

It is hoped that once the condition in the country is settled, rapid strides in nutrition will be made.

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PAPER PARTITION CHROMATOGRAPHY

Advances in nutrition, as in most fields of science, usually follow upon the development of suitable analytical methods. One of the more dramatic developments of the past few years has been that of paper partition chromatography, which is obtaining application among many classes of substances of interest to nutritionists as well as biochemists in general.

Paper partition chromatography is a

special type of chromatography, which takes advantage of differences in mobility of structurally related compounds when these are dissolved in a solvent which advances along a strip of paper due to capillary action. The essentials of the apparatus were described by R. Consden, A. H. Gordon, and A. J. P. Martin (*Biochem. J.* **38,** *224 (1944)*). They consist in general of a hanging filter paper strip, the upper end of which is immersed in a trough containing the solvent saturated with water. The strip hangs in an airtight chamber which is maintained in an atmosphere saturated with the solvent and water. The sample to be partitioned is placed as a spot on the upper part of the filter paper strip to be extracted by the advancing solvent.

The use of solvents partly miscible with water has been recommended by these authors. When used with amino acids, for example, the separation of the various amino acids depends upon the differences in partition coefficient between the moving phase and the water-saturated cellulose, rather than upon differences in adsorption by the cellulose; this favors the production of sharply defined spots on the paper, whereas if water or a miscible solvent-water combination is used, the spots tend to become longer and less distinct, possibly due to actual adsorption on the filter paper. Identification of the spots is usually accomplished through the development of color; with amino acids this is done by spraying the paper with ninhydrin.

Since the progress of the solutes depends upon their partition coefficients between the other solvent and the water-saturated paper, it is to be expected that the distance traversed by each solute will always be in constant ratio to the distance covered by the advancing front of the solvent. This is borne out by observation. For solutes whose partition coefficients are nearly alike, the distances traversed will be nearly the same, and a longer course is necessary to separate these. The separation of leucine and isoleucine, for example, is relatively difficult, for (with phenol as the partitioning liquid) the ratio of advance of the amino acids to the advance of the phenol is 0.85 and 0.86, respectively. In other words, when the solvent has traversed a 100 cm. strip, the two amino acids have reached 85 and 86 cm.

Since the authors claim reproducibilities of only 2 to 4 per cent, it is obvious that such closely related compounds may not be separated by a single partitioning experiment. Nevertheless, phenol provides excellent separation of most of the other amino acids. Thus, the above ratios (called R_F values) range from 0.91 for proline to 0.15 for aspartic acid. A good "spectrum" is obtained for twenty-three amino acids; only proline, phenylalanine, norleucine, isoleucine, leucine, tryptophan, norvaline, and methionine lie close together, with R_F values of 0.91, 0.89, 0.88, 0.86, 0.85, 0.83, 0.81, and 0.80, respectively.

To obtain further separation of compounds with similar R_F values, the authors resorted to the use of other solvents and a large paper sheet, so that at the end of the experiment the chromatogram could be turned 90° and a new chromatogram developed. With *o*-cresol as the new solvent, the above seven amino acids gave R_F values of 0.69, 0.81, 0.72, 0.58, 0.61, 0.65, 0.45, and 0.58, respectively. (The value of 0.58 for both isoleucine and methionine would not cause difficulty, since the corresponding values in phenol were 0.86 and 0.80; the new paths for these compounds would thus be parallel.) According to the authors, even leucine and isoleucine can be recognized in a two-dimensional chromatogram, especially if known mixtures of these acids were developed simultaneously on another portion of the paper.

Probably the most attractive feature of paper partition chromatography is the fact that only small amounts of material are required. It is claimed that a com-

plete separation of the common amino acids can be made with 300 to 500 micrograms of protein hydrolysate. In addition, the method is rapid; a two-dimensional chromatogram can be developed in two to six days. A considerable saving of time and material is thus afforded, even over microbiologic assays, although the latter possess the added advantage of providing quantitative measurements.

A publication by H. B. Bull, J. W. Hahn, and V. H. Baptist (*J. Am. Chem. Soc.* **71,** *550* (*1949*)) offers promise of extension of paper chromatography into quantitative determination of amino acids. The special features of this method include the selection of filter paper (Schleicher and Shull number 507) sufficiently dense to provide a slow flow of the solvent (phenol), so as to provide sharp resolution of the amino acids; uniformity of light transmission throughout the paper; and freedom from impurities which would produce extraneous colors with ninhydrin. Eighty per cent phenol-water mixtures were employed rather than the saturated mixtures of Consden in order to minimize the effect of temperature changes.

After development of the chromatogram (forty-eight hours) and the color bands with ninhydrin, the filter paper strips (7.2 mm. wide and 70 cm. long) were mounted on glass slides 70 cm. long and 4 cm. wide. These were blacked out except for a groove 7.2 mm. wide in which the filter strip was placed. The glass strip was then passed between an illuminated slit and a phototube. The percentage light transmission was measured throughout the entire length of the strip at 5 mm. intervals, and compared with a "blank" strip which had been carried through the entire procedure without the addition of amino acids.

The percentage transmission was then plotted on semi-log graph paper against the distance along the filter paper strips. The areas between the plotted curve and 100 per cent transmission were measured with a planimeter in square inches. These areas were found to be a simple function of the concentration of the amino acids, over a range of approximately 0.7 to 13.5 micrograms of each amino acid present. The probable error over this range was ±8.8 per cent. The areas were then expressed by an algebraic equation as a function of the concentration, which was valid for arginine, serine, valine, glutamic acid, leucine, threonine, alanine, and lysine. However, aspartic acid, phenylalanine, methionine, and glycine did not conform to the standard equation. It was felt that this was due chiefly to the observed failure of these acids to develop the maximum color with ninhydrin, in contrast to the others which were reported to develop maximum color intensity after heating at 90°C. for five minutes, followed by steam heating for five minutes. No statement was made concerning the behavior of the other amino acids commonly found in proteins, and they were presumably not studied.

Using the amino acids which conformed to the standard equation described above, these authors assayed known mixtures by the new technic. Quantities of 3.38 micrograms each of lysine hydrochloride, arginine hydrochloride, threonine, alanine, valine, and leucine were mixed and subjected to analysis. The quantities found were: lysine hydrochloride 3.78, alanine 2.84, valine 3.51, and leucine 3.32. The arginine and threonine curves overlapped considerably, so that only the sum of the two compounds could be given; this was found to be 0.043 micromole, compared with the known value of 0.048. It would appear from these results that quantitative determinations of amino acids after paper partition may be feasible, although further work is needed to obtain separate values for the overlapping amino acids.

Although the bulk of paper partition chromatography research has been applied in amino acids, initial studies have also been made with other classes of compounds.

S. M. Partridge (*Biochem. J.* **42,** *238, 251 (1948)*) has applied this technic to the qualitative analysis of sugars, using a two-dimensional system with phenol and *s*-collidine as the developing solvents, and ammoniacal silver nitrate for development of color. R_F values were reported for twenty-two sugars and their derivatives, including uronic acids, amino sugars, inositol, and ascorbic acid. The method was generally operative over the range from 5 to 40 micrograms of each sugar, although it was stated that glucose could be readily detected in smaller amounts. The method has been applied to the detection of sugars in egg white, apples, the fetal blood of sheep, and the hydrolytic products of the specific polysaccharide of Bact. dysenteria and the blood group A substance from hog gastric mucin.

E. Vischer and E. Chargaff (*J. Biol. Chem.* **168,** *781 (1947)*) applied the method to purine identification, using a 3:1 quinoline-collidine mixture, and developing the color with mercuric nitrate in nitric acid, followed by aqueous ammonium sulfide. Detection of as little as 5 micrograms of purine was possible, and excellent separation was effected; R_F values for guanine, adenine, and xanthine were 0.26, 0.39, and 0.61, respectively. The study was extended to include pyrimidines by the same authors (*Ibid.* **176,** *703 (1948)*) wherein R_F values for cytosine, uracil, and thymine were found to be 0.21, 0.74, and 0.84, respectively, in these solvents. Amounts of the above compounds (except xanthine) ranging from 5 to 40 micrograms were separated satisfactorily.

C. E. Carter (*Abstracts, Am. Chem. Soc., 115th Meeting, 25C (1949)*) has likewise reported the successful paper chromatography of purine and pyrimidine derivatives of yeast nucleic acid. Using butanol-aqueous urea, followed by isoamyl alcohol-aqueous inorganic phosphate, he has obtained resolution of all of the degradation products of yeast nucleic acid except cytidilic and uridylic acids. Finally, R. Consden (*Nature* **162,** *359 (1948)*), in a review dealing with paper chromatography, has reported the separation of anthocyanin pigments from petals, organic acids, adrenalin-like substances, creatine and creatinine, vegetable tannins, and penicillins.

The number of important contributions which may be made through paper chromatography to the study of the minor constituents of tissues appears very great. Aside from the usual benefits to be derived from new microtechnics, Consden points out that this one may be used successfully to study the sequence of amino acid residues in proteins and polypeptides. Simple peptides, which may be produced through partial hydrolysis of larger molecules, may be separated satisfactorily by one- or two-dimensional chromatography (R. Consden, A. H. Gordon, and A. J. P. Martin, *Biochem. J.* **41,** *590 (1947)*).

The chromatograms are developed in duplicate or larger; one is treated with ninhydrin and serves as a guide to the cutting of the other. The material may then be washed from the paper, according to the authors, and again subjected to chromatography, after a preliminary hydrolysis or deamination, to determine the constituent amino acids. By the use of this technic these workers have established the sequence of amino acids in gramicidin-S and in tyrocidin. Applications of this technic in the determination of partially degraded proteins or polypeptides appear especially promising.

MAGNESIUM DEFICIENCY

The indispensability of magnesium in diets was accepted long before the actual demonstration of its essential character, because of its widespread occurrence in

plant and animal tissues. One of the first experimental studies that gave evidence of specific manifestations of magnesium deficiency other than inhibition of growth was reported in 1932 by H. D. Kruse, E. R. Orent, and E. V. McCollum (*J. Biol. Chem.* **96,** *519 (1932)*). These workers described in detail the abnormalities that occur with astonishing rapidity in young rats on diets very low in magnesium (0.18 mg. per cent).

Marked variations in heart rate and in the distribution of blood were followed by hyperexcitability and convulsive seizures. The majority of animals died during the recovery phase after the first seizure. It was noted that the hissing sound of an air blast was particularly effective in causing the onset of convulsions. Animals that survived the first seizure and lived for longer periods showed loss of hair, lesions of the skin, and other general symptoms of nutritive failure. In later experiments these authors found a progressive decrease in serum magnesium during the course of the deficiency and a subsequent increase in the cholesterol fraction of blood (*J. Biol. Chem.* **100,** *603 (1933)*).

The deficiency syndrome ascribed to lack of magnesium is not limited to the rat. It has been reported in dogs (E. R. Orent, H. D. Kruse, and E. V. McCollum, *Am. J. Physiol.* **101,** *454 (1932)*), and in calves (C. W. Duncan, C. F. Huffman, and C. S. Robinson, *J. Biol. Chem.* **108,** *35 (1935)*).

It is probable that the "grass tetany" described by B. Sjollema and L. Seekles (*Biochem. Z.* **229,** *358 (1930)*) and discussed by H. D. Kruse, E. R. Orent, and E. V. McCollum (*J. Biol. Chem.* **100,** *603 (1933)*) is complicated, at least, by a deficiency of magnesium. Little is known of the human requirement for magnesium (*Nutrition Reviews* **4,** *195 (1946)*) but it is reasonable to assume that a dietary lack of this element would cause abnormalities of the type observed in animals.

Effects somewhat similar to those produced in rats, dogs, and cattle have also been noted in young rabbits (H. O. Kunkel and P. B. Pearson, *J. Nutrition* **36,** *657 (1948)*). Weanling rabbits showed hyperexcitability, convulsions, retardation of growth, and decrease in blood magnesium within three to six weeks on a diet deficient in this mineral. As in the case of rats, unusual noises were sufficient to cause alarm and convulsive seizures. Magnesium levels dropped from 4.4 mg. per cent for whole blood and 1.6 mg. per cent for plasma to 3.1 and 1.0 mg. per cent, respectively, after eight weeks. No change in serum calcium was found. Loss of weight and extreme emaciation preceded death. Gross examination of the kidneys revealed a mottled, bloody appearance. The vasodilatation and hyperemia that were prominent early symptoms in rats were not observed.

Kunkel and Pearson have also determined the magnesium requirement of the rabbit, using the blood level as the index. No evidence of deficiency was found when the diet contained 32.5 mg. per cent of magnesium but a decreased blood level occurred on a diet containing 25 mg. per cent, and audiogenic convulsions on a diet containing 17.5 mg. per cent. These authors have reported previously that a dietary level of 20 mg. per cent maintained a normal blood magnesium level in rats (H. O. Kunkel and P. B. Pearson, *Arch. Biochem.* **18,** *461 (1948)*).

In earlier studies, E. V. Tufts and D. M. Greenberg (*J. Biol. Chem.* **122,** *715 (1938)*) found that a dietary level of 5 mg. per cent was a borderline level for good growth and that females on this diet gave birth to young of normal weight and of normal magnesium content. However, the mother's milk appeared deficient in magnesium inasmuch as the suckling young showed the characteristic signs of magnesium deficiency and the expected increase in body magnesium did not occur.

The hyperexcitability and tetanic convulsions of magnesium deficiency are be-

lieved to represent a specific disorder, although generally related to the convulsive seizures due to low serum calcium (H. D. Kruse, M. M. Schmidt, and E. V. McCollum, *J. Biol. Chem.* **106,** *553 (1934)*). It is possible that the deficiency syndrome may include metabolic changes involving the role of the magnesium ion in phases of metabolism not directly concerned with calcium.

Magnesium is believed to constitute a part of the thiamine-containing enzyme, carboxylase (D. E. Green, D. Herbert, and V. Subrahmanyan, *J. Biol. Chem.* **138,** *327 (1941)*), and it may have additional functions of this type. Indeed, thiamine and magnesium deficiencies have certain points of similarity. Although present evidence indicates that magnesium is held tenaciously by the tissues, other than blood, of animals on low magnesium diets, the *in vitro* study of the specific enzymatic activity of these tissues should provide important data. The results of Kunkel and Pearson add the rabbit to the list of experimental animals that may be used for this purpose.

ANTIDIURETIC SUBSTANCE IN THE URINE OF PATIENTS AND ANIMALS WITH DAMAGED LIVERS

Much work has been devoted to the study of the causes of edema formation in patients with cirrhosis of the liver. D. Adlersberg and C. L. Fox (*Ann. Int. Med.* **19,** *642 (1943)*) found an abnormal retention of water when water tests were performed on patients with severe parenchymatous liver disease. They also observed abnormal water tolerance tests in dogs whose livers were damaged by phosphorus and histamine. H. Shay, R. Kolm, and S. S. Fels (*Gastroenterology* **4,** *257 (1945)*) have reported the occurrence of abnormal water tolerance tests in rats fed a high fat diet.

E. P. Ralli, J. S. Robson, D. Clarke, and C. L. Hoagland (*J. Clin. Invest.* **24,** *316 (1945)*) were unable to establish any relation between the level of serum albumin and the presence or absence of edema in cirrhotic patients. Because of the decreased urine volume noted in edematous cirrhotic patients they decided to examine the urine of these patients for antidiuretic hormone activity. Using the assay method of J. H. Burn (*Quart. J. Pharm. Pharmacol.* **4,** *517 (1931)*) they found the urinary antidiuretic activity of these subjects to be increased. The antidiuretic assay was found to be proportional to the degree of edema and ascites, being only slightly above the control normal level in the cirrhotic patient who was free of edema.

S. H. Leslie and E. P. Ralli (*Endocrinology* **41,** *1 (1947)*) have found that rats fed a high fat-low protein diet showed a decreased output of urine during water tolerance tests, and they observed that the urine from such rats had antidiuretic activity.

C. A. Hall, B. Frame, and V. A. Drill (*Endocrinology* **44,** *76 (1949)*) have published confirmation of the finding of an antidiuretic factor in the urine of cirrhotic patients. The urine antidiuretic activity was assayed by the method of G. C. Ham and E. M. Landis (*J. Clin. Invest.* **21,** *455 (1942)*). The cirrhotic patients studied all had ascites. The twenty-four hour urinary antidiuretic effect in these subjects was comparable to that produced by 5 milliunits of pituitrin. However, these workers found that urine extracts prepared from rats with dietary-induced liver damage or without liver damage all showed antidiuretic activity. No difference in the intensity of the antidiuretic effect was noted in the absence or presence of liver injury. Also normal water tolerance tests were obtained in rats that had dietary-induced fatty and fibrotic livers. These workers felt that differences in pretreatment of the animals in preparation

for water tolerance tests might explain the discrepancies reported in the literature.

In general, the studies on urinary antidiuretic activity in patients, too, should evaluate more carefully the factor of water ingestion prior to and during the time of urine collection. Many ill patients have an aversion for fluid as well as food and a decreased fluid ingestion would require increased antidiuretic activity to affect renal conservation of the limited water ingested.

UTILIZATION OF GLYCINE FOR HEME FORMATION

Heavy nitrogen (N^{15}) has been proved to be an excellent material for labeling nitrogen-containing compounds such as glycine, glutamic acid, proline, lysine, and ammonia (*Nutrition Reviews* **5,** *156 (1948)*). Through the use of this procedure for labeling the above nitrogen-containing compounds, glycine was found to be used more efficiently in the formation of the heme portion of the hemoglobin molecule than the other sources of nitrogen tested. Presumably the glycine molecule was used directly, whereas the nitrogen of other compounds may have been used indirectly.

I. M. London, D. Shemin, and D. Rittenberg (*J. Biol. Chem.* **173,** *797 (1948)*) studied the ability of the human red blood cell in cases of sickle cell anemia to synthesize heme *in vitro*. The whole blood of subjects with sickle cell anemia was incubated with glycine labeled with heavy nitrogen. After incubation for twenty-four hours, the heme was isolated as hemin and found to contain a significant amount of heavy nitrogen. The reticulocyte counts of the sickle cell anemia blood samples ranged between 15 and 20 per cent.

When blood samples from normal subjects, and from subjects with other blood disorders, such as sickle cell trait without anemia, hypochromic anemia, pernicious anemia, and congenital hemolytic jaundice were incubated with labeled glycine, no ability to synthesize heme from glycine was observed. In most cases, the reticulocyte counts were comparable to those characteristic of the samples from subjects with sickle cell anemia.

The data clearly demonstrated that a mechanism existed in the blood of subjects with sickle cell anemia which permitted the synthesis of heme from glycine to be carried out *in vitro*. As yet, no active mechanism of this type has been demonstrated for human blood in normal or other pathologic states. The presence of reticulocytes in the blood samples from patients with sickle cell anemia seemed to be eliminated as a possible cause of the *in vitro* synthesis of heme.

This problem has been continued by D. Shemin, I. M. London, and D. Rittenberg (*J. Biol. Chem.* **173,** *799 (1948)*). Since hemoglobin in the non-nucleated mammalian red blood cells has normally been produced prior to loss of the nucleus, the ability of the nucleated red blood cell from the peripheral blood of the duck to synthesize heme from glycine *in vitro* was investigated. Red blood cells of the duck, suspended in whole blood or in saline, were incubated in the presence of glycine, labeled with heavy nitrogen for periods of from two to twenty-four hours at a temperature of 37°C. In all cases, there was an appreciable amount of heavy nitrogen in the heme which was isolated from hemoglobin at the end of the incubation period.

In another series of experiments, K. I. Altman and co-workers (*J. Biol. Chem.* **176,** *319 (1948)*) studied the effect of administration of glycine labeled with radioactive carbon in the alpha carbon position upon the radioactivity of the hemin residue and of the globin component of hemoglobin.

Four normal adult rats were fed labeled glycine in a single dose in aqueous solution

by stomach tube. The total dose administered was 2 microcuries, that is, approximately 1 microcurie per 100 g. of body weight. At varying periods, after glycine feeding, the rats were anesthetized and bled as completely as possible from the carotid artery. Hemoglobin was isolated by crystallization, from aliquots of the hemoglobin, crystalline hemin was prepared and globin isolated. Globin preparations were carefully washed with acetone and ether and were found on spectrographic analysis to contain no hemin. Organic material in the samples was converted to carbon dioxide by wet digestion. The carbon dioxide produced was introduced into an ionization chamber for determinations of the amount of radioactive carbon dioxide present.

In addition, 3 rats were made anemic by giving 24 mg. of phenylhydrazine hydrochloride by stomach tube on two alternate days. The rats were then allowed to enter the recovery period in the course of which the reticulocyte count attained a level of 15 to 20 per cent, at which time glycine labeled with radioactive carbon in the alpha position was administered to the 3 rats. There was an appreciable amount of radioactive carbon in the hemin and in the globin prepared from both normal and phenylhydrazine-poisoned rats. On the basis of calculations of the total amount of circulating blood, 0.49 per cent of the administered dose was present in the hemin and 1.42 per cent in the globin at the end of the first day. By the sixth day these values had increased to 0.66 per cent and 2.57 per cent, respectively, in the normal rats.

The rats made anemic by phenylhydrazine hydrochloride administration were found to be synthesizing hemoglobin at a more rapid rate and, therefore, it is not surprising that the hemoglobin in these animals contained considerably higher concentrations of radioactive carbon than that of the normal rats. When the radioactive carbon content of hemoglobin was calculated by assuming a molecular weight of 68,000 and that 1 molecule of globin is capable of combining with 4 molecules of hemin, the theoretical calculated value was found to agree closely with the measured amount of radioactivity. This was true both in normal and in anemic rats. The radioactive carbon activity per glycine residue was approximately one and a half times as high for hemin as for globin, suggesting that the rate of hemin synthesis was slightly faster than the rate of globin synthesis.

In addition, the effect of administration of glycine labeled with radioactive carbon in the carboxyl position has been tested in a dog weighing 10 kg. and made anemic by bleeding (M. Grinstein, M. D. Kamen, and C. V. Moore, *J. Biol. Chem.* **174,** *767 (1948)*).

Over a period of three days, 100 mg. of glycine labeled with radioactive carbon were given by stomach tube. Samples of blood were taken on the eleventh, fourteenth, twentieth, thirty-seventh, and fifty-second days following administration. Crystalline protoporphyrin methyl ester and globin were obtained and the radioactivity measured with a thin wall Geiger-Müller counter. No radioactivity was observed in the protoporphyrin. However, the globin showed an activity that remained constant throughout the period of observation.

A rat weighing 100 g., which had been kept on a protein-free diet, was given, in four portions during a period of three days, 200 micrograms of the same labeled glycine. Twenty days later the animal was bled, crystalline protoporphyrin methyl ester was prepared, and again the radioactivity determined. Therefore, it was seemingly proved that the carboxyl carbon of glycine could not be used in the biosynthesis of protoporphyrin but that it was incorporated into the globin molecule.

The above studies considered together present a general survey of the utilization of various portions of the glycine molecule

in the production of heme. Apparently glycine was decarboxylated and the residue used for synthesis of the heme molecule with the carboxyl carbon being of no value in this synthesis but being utilized in the production of the globin fraction of hemoglobin. The nitrogen and alpha carbon atoms were readily utilized in the synthesis of heme.

The investigations with red blood cells from the blood of patients with sickle cell anemia indicated that sickle cells have maintained an ability to synthesize heme and hemoglobin *in vitro* long after red blood cells from normal subjects had lost that ability. In this regard, the sickle cell anemia red blood cells were similar to the nucleated red blood cells from the duck. The evidence presented indicates that the ability to synthesize heme probably was not due to the presence of reticulocytes among the sickle cells.

CONCENTRATIVE ASSIMILATION OF AMINO ACIDS AND IMBALANCE IN AMINO ACID NUTRITION

Instances of growth retardation due to excesses of one or several amino acids in the diet have been reported with increasing frequency. An interesting example is the decrease in the protein efficiency of diets for rats when methionine supplementation is brought to 2 per cent (C. F. Kade and J. Sheperd, *Federation Proceedings* **7,** *291* (*1948*)).

Much attention has fallen upon the increased requirement for tryptophan or niacin on diets high in gelatin or corn products. A new idea is introduced by the studies of H. N. Christensen and co-workers (Christensen, J. A. Streicher, and R. L. Elbinger, *J. Biol. Chem.* **172,** *515* (*1948*); Christensen, *Federation Proceedings* **8,** *191* (*1949*)), as to the way in which high intakes of one or several amino acids may interfere with amino acid assimilation.

Christensen and his collaborators studied the distribution of glutamine, of glycine, and of all other amino acids ("residual" amino acids) measured collectively by ninhydrin, between the cells of skeletal muscle and of liver, and the plasma of the guinea pig. The glycine levels were highly variable, but the variations occurred together in the three tissues, so that relatively constant distributions were maintained. The glycine of the liver cells was, as an average, thirty-three times that of the extracellular fluid, that of the muscle cells, nine times.

The distribution of the "residual" amino acids also appeared to be constant from animal to animal, whereas glutamine was distributed in a variable fashion. The effects of feeding guinea pigs various amino acids were then investigated. Twenty millimoles of an amino acid were fed per kilogram of animal weight in three hourly doses (13.3 millimoles in the first dose, 3.3 in the other two). After another hour the animal was killed and the amino acid distribution studied.

Most of the amino acids in the L-forms produced small to moderate increases of the plasma amino acid levels. Glycine, histidine, methionine, and proline, as well as the DL-forms of several amino acids, led to high plasma amino acid concentrations. High glycine concentrations were observed in association with greatly diminished cellular levels of "residual" amino acids. Conversely, whenever high plasma amino acid nitrogen concentrations were produced by feeding an amino acid other than glycine, a roughly proportional fall in the distribution ratio for glycine between cells and extracellular fluid was observed.

High plasma levels of an amino acid appeared to interfere with the cellular activity for concentrating other amino acids. In one

case, after feeding proline, the distribution coefficient for liver glycine fell to one-fourth its usual value, with the plasma glycine nitrogen rising to 7 mg. per cent.

The investigators interpret these interactions as competitive phenomena, based upon the structural features shared by the amino acids. They suggest that the loss of certain cellular amino acids as a result of excessive plasma concentrations of other amino acids may decrease the rate of protein synthesis relative to the rate of protein breakdown. They propose that the cell must have present at some limiting concentration each amino acid, essential or nonessential, which is to be built into a protein molecule, before the protein can be formed. Although this postulate is not easily controverted, unfortunately, neither the values for the limiting concentrations nor the forms in which the amino acids must be present are known.

Feeding of glutamic acid, in contrast, led to decreased plasma levels of each of the three categories of amino acids measured. This effect was also observed in the dog. Analyses of the tissues indicated that the decrease in plasma concentration was due to an increased uptake of amino acids by the cells of liver and muscle; that is, the concentrating activity appeared to be increased. According to this interpretation of the plasma concentration of amino acids, decreased levels may result from an intensification of the concentrative assimilation by the cells; an elevated plasma level may occur when the concentration process is inhibited.

The plasma amino acid concentration is considered a result of the simultaneous concentrative activities of various tissues. This view appears to agree with the observation of a rise of tissue amino acid levels and a fall of plasma levels after adrenalectomy (F. Friedberg and D. M. Greenberg, *J. Biol. Chem.* **168,** *405 (1947)*) as well as with the elevation of plasma amino acids by adrenocorticotropic hormone, and their depression by pituitary growth hormone (C. H. Li, I. Geschwind, and H. M. Evans, *Ibid.* **177,** *91 (1949)*). The depression of plasma amino acid concentrations in pregnancy, when the placenta assumes a large concentrative activity, is presented as another illustration (H. N. Christensen and J. A. Streicher, *Ibid.* **175,** *95 (1948)*).

The nature of the concentrating activity for amino acids was somewhat clarified by studies of the isolated diaphragm. Excised rat diaphragm maintained its glycine level at about four or five times the fluid glycine concentration, whether the fluid concentration was varied upwards or downwards, in artificial mediums. A sodium chloride-sodium bicarbonate medium containing 2.5 millimolar calcium chloride and 20 millimolar sodium pyruvate, or rat plasma fortified with pyruvate, served to maintain the concentrating activity for several hours at 37°C.

At lower temperatures much smaller distribution ratios were obtained. The total glycine content of the system increased only slightly, whereas there were striking changes of the glycine distribution. Rapid dissipation of the glycine gradient occurred in the absence of oxygen, or in the presence of 80 millimolar potassium, 3 millimolar cyanide, 25 millimolar arsenite, or 2,4-dinitrophenol at concentrations as low as 5×100^6 millimolar.

The stimulating effect of pyruvate and the inhibiting effects of dinitrophenol were also observed for the concentrating of amino acids as measured collectively by ninhydrin, using rat plasma as the suspending fluid. Apparently the concentrative assimilation of amino acids by the cell depends for energy upon exergonic, i.e., energy-producing, metabolic reactions. A similar conclusion was reached for certain bacterial species by E. F. Gale (*Bull. Johns Hopkins Hosp.* **83,** *119 (1948)*).

The inhibitory effects of various amino acids upon glycine assimilation were then studied with the isolated diaphragm. Of

the alpha-amino acids tested all inhibited, at 21 millimolar concentration, the maintenance of the distribution coefficients *in vitro*. Two alpha-methyl-alpha-amino acids which are not readily degraded by the animal organism were included. D- and L-forms were equally effective, and the nature of the side chain as to size and charge appeared to be unimportant. Amino acids with the amino group in other than the alpha position were without effect. The inhibitory effect of valine was eliminated when an N-formyl group was introduced. Whether the high amino nitrogen concentration was due to one or a number of amino acids, strong inhibition was observed.

These studies show that the amino acid pattern of the cell can be upset by high amino acid levels in the extracellular fluid, with the cellular concentrations of some amino acids falling while others are rising. Sustained small increases in the level of one or several amino acids due to excesses in the diet, or brief but large accumulations during intravenous infusions, might handicap cellular functions dependent upon amino acid levels, especially, perhaps, protein synthesis. It would be interesting to know whether the formation of niacin from tryptophan is particularly sensitive to decreased cellular concentrations of tryptophan.

MODIFICATION OF FAT ABSORPTION IN THE DIGESTIVE TRACT BY USE OF AN EMULSIFYING AGENT

Faulty absorption of fats from the intestinal tract occurs clinically not only in the uncommon disorder, sprue, but also in certain disorders of the pancreas, infections of various parts of the gastrointestinal tract, and after partial removal of the stomach. Whereas normally the average fat content of the stools comprises not more than 4 per cent of the ingested fats (E. E. Wollaeger, M. W. Comfort, J. F. Weir, and A. E. Osterberg, *Gastroenterology* **6,** *93 (1946)*; Wollaeger, Comfort, and Osterberg, *Ibid.* **9,** *272 (1947)*), under the conditions named above, the fecal lipid content may run as high as 40 to 50 per cent of the dietary intake (C. M. Jones, P. J. Culver, G. D. Drummey, and A. E. Ryan, *Ann. Int. Med.* **29,** *1 (1948)*).

In these conditions, nutritional improvement has been obtained by various means with varying degrees of success. Thus, in sprue, the use of liver extract or of folic acid in optimal amounts may result in increased intestinal absorption. Nevertheless, it is rare in so-called nontropical sprue to modify the flat vitamin A tolerance curve so characteristic of this condition. In pancreatic fibrosis, potent pancreatic extracts occasionally may be of great clinical benefit. In patients with a major loss of absorptive surface secondary to an inflammatory process or to short-circuiting operations, overfeeding may be the only therapeutic measure to afford help.

Because none of these therapeutic measures is wholly satisfactory, Jones, Culver, Drummey, and Ryan (*loc. cit.*) undertook a study of the possible value of emulsifying agents in facilitating the absorption of fat. R. J. Dubos (*Proc. Soc. Exp. Biol. Med.* **58,** *361 (1945)*) had found that an emulsifying agent used in industry to effect an even distribution of flavors, perfumes, and lipoid substances in an aqueous or lipoid medium, achieved such a homogeneous dispersion of tubercle bacilli in an aqueous culture medium that individual colonies could be grown in place of the usual growth in the form of a pellicle. This led Jones and co-workers to inquire whether a similar agent when mixed with food might provide a more homogeneous and finer emulsification of dietary fats and a better dispersal of lipoid substances when presented to the intestinal

mucosa for absorption. They chose polyoxyethylene sorbitan monooleate (PSM, marketed under the trade name Tween 80), a substance which is prepared by mixing sorbitan monooleate with ethylene oxide in the ratio of 20 mols of the latter per mol of the former.

Long-term feeding experiments by J. C. Krantz (*unpublished*) have shown no toxic manifestations following feeding of PSM to animals over several generations. Unpublished studies have also demonstrated that at least the polyoxyethylene fraction of PSM is excreted quantitatively in the urine and stools. Thus there is little likelihood of oxalic acid poisoning from the polyoxyethylene component. Furthermore, urinary studies for oxalate content in patients on PSM therapy have indicated no increase in oxaluria. The only symptom attributable to use of this preparation has been the rare manifestation of increased bowel activity. In human beings, as much as 15 g. have been fed daily for a period of months without any untoward symptoms and without any evidence of toxicity as measured by blood studies or liver and renal function tests.

In a series of 16 normal subjects the investigators carried out serum vitamin A determinations on specimens taken fasting and at intervals of three, five, and seven hours after ingestion of 200,000 units of vitamin A ester in fish liver oil. Two weeks later the tests were repeated, but at this time 2 g. of PSM were added to the capsules containing the fatty solution of vitamin A. The peak level of vitamin A obtained in the serum was essentially the same in the control curves and following the use of the emulsifying agent, as was to be expected, inasmuch as 96 per cent or more of ingested fat is absorbed by normal individuals. However, the peak of absorption occurred at about three hours as compared with five hours when the subjects were not given PSM. This suggests that absorption was accelerated.

Similar studies were also carried out in patients suffering from such conditions as subtotal gastrectomy, sprue, pancreatic fibrosis, and regional enteritis. In these subjects, control observations demonstrated a striking interference with fat absorption as shown by low or flat vitamin A tolerance curves as well as by the presence of an abnormally high fat content in the stool and by inability to gain weight on ordinary diets. The addition of 2 g. of PSM to the test dose of fat soluble vitamin A resulted in an easily demonstrable increase in vitamin A absorption as compared with the original control observations. This increase amounted, in some instances, to as much as 400 to 500 per cent of the control peak figures.

Controlled metabolic studies were also made in a group of patients with nutritional difficulties secondary to subtotal gastrectomy performed for duodenal ulcer. In a group of 4 patients, studies were made on stool fats by the method of F. S. Fowweather (*Brit. J. Exp. Path.* **7**, 7 (*1926*)) under control conditions and following use of various substances that might conceivably increase absorption of fat from the small bowel. No important reduction in percentage of fecal fat occurred after use of such substances as desoxycholic acid, folic acid, pancreatic extract, hydrochloric acid, and crude liver extract.

Similar observations were made on 4 other patients with subtotal gastrectomies before and after use of PSM. All 4 of these patients were badly nourished and had difficulty in doing more than maintain their weight even with a very adequate caloric intake. In 2 cases the amount of fat lost in the stool was 12.4 and 14.6 per cent of the ingested fat respectively. After adequate control periods on an absolutely constant diet, PSM was added with the meals in doses of 1.5 g. three times a day. In each case, after addition of this emulsifying agent, there was a reduction in fecal fat. In one of the patients the amount of fat in the diet was subsequently doubled. When the daily

fat intake was raised from 98 to 183 g. the percentage of fat lost in the stools remained essentially the same, although the total amount lost was practically double. At this point PSM was again added to the diet. Its use resulted in a measurable reduction in stool fats even though the amounts of dietary fat had been practically doubled.

Another patient was studied prior to operation. The percentage of fat lost in the stool during this preoperative control period amounted to 3.2 per cent of 100 g. fat intake. Twenty-eight days after a subtotal gastrectomy was performed, the patient was able to take only 40 g. of fats; of this, 10.8 g. or 27 per cent was lost in the stools. When, six months later, the patient had become reasonably well adjusted to the operative procedure, he was given a full diet of 3000 calories, containing 100 g. of fat. At this time his weight was still approximately 13 kg. under his normal weight and only 1.7 kg. greater than his immediate preoperative weight. On this diet the stool fats amounted to 14.6 per cent of the fat intake. When PSM was added, the fecal fat dropped to a figure almost identical with that observed in the original preoperative control period.

Another type of study was carried out on a young woman who had been treated for sprue for a year and a half. Her diet contained approximately 2400 calories and consisted of 215 g. of carbohydrate, 90 g. of protein, and 130 g. of fat. Folic acid was administered in daily doses of 15 mg. by mouth. On this regimen, the clinical symptoms of the disease were partially controlled, but over the entire nine months' period there was essentially no gain in weight. Several determinations of stool fat showed a loss in fecal lipids of approximately 22 g. per day. At the end of nine months, 1.5 g. of PSM were added with each of the three meals. There followed a slow steady gain of 21 pounds in weight and later, when folic acid was omitted, there was no diminution in weight gain. The clinical symptoms during this period were almost completely controlled. The steady gain in weight was accompanied by progressive diminution of the amount of fat lost in the stools. The final determination of fecal fat loss was approximately 7 g. per day.

On the basis of these observations it appears that the agent, polyoxyethylene sorbitan monooleate, actually has an important influence on the absorption of fat. This is perhaps accomplished through its ability to lower surface tension. The effect primarily may be that of a substance capable of modifying opposing interfaces with resulting improvement of emulsification or dispersion. Such an agent, by lowering surface tension, in the case of dietary fats, probably increases the total surface area of lipoid material to be presented to the intestinal villi by reducing the size of the fat globules.

Since fecal fat excretion varies considerably from period to period, it might be wise in future studies to use control periods both before and after the agent under investigation is tested. This precaution might overcome just criticism when one is treating a disease, such as nontropical sprue, which is known to have spontaneous and prolonged remissions.

PROTOGEN: A NEW GROWTH FACTOR

Among the first protozoa to be grown in cultures free of bacteria were Tetrahymena. These organisms have been grown successfully on purified mediums and the amino acid requirements of the protozoa investigated. Further, it has been shown that the organism requires pteroylglutamates, purines, and pyrimidines for growth. In addition, it was found that these organisms require an unidentified water soluble factor

which has been termed "Factor II." These several studies were summarized in an earlier review (*Nutrition Reviews* **6,** *38 (1948)*).

Investigations now reported by E. L. R. Stokstad *et al.* (*Arch. Biochem.* **20,** *75 (1949)*) indicate that the Tetrahymena Factor II consists of at least two components, for one of which they propose the name "protogen," in view of the essential nature of the factor for the growth of a protozoon.

The investigators from the Lederle laboratories used Tetrahymena geleii as the test organism. The basal medium contained hydrolyzed casein, DL-tryptophan, hydrolyzed yeast nucleic acid, dextrose, salts, and the well identified B-complex vitamins (including pteroylglutamic acid and biotin). Inoculation of an entire batch of medium was made, and separate aliquots were delivered to individual flasks for study. This technic was necessitated by the tendency of the free-swimming T. geleii to stratify in the pipet during inoculation of smaller quantities. Incubation was for forty-four hours at 25°C.

Liver-press cake, a source of Factor II, was separated into two components as follows: the press cake was digested with papain, taken up in water, adjusted to pH 3.0, and freed of proteinaceous precipitate. The filtrate therefrom was adjusted to pH 2.0 and precipitated with an excess of silver nitrate. This precipitate was then freed of silver by hydrochloric acid and the resulting silver-free fraction termed "Acid Silver Precipitate (IIA)." The filtrate from the pH 2.0 silver precipitation was then brought to pH 4.0 and a second silver precipitate collected. This was freed of silver, as before, and the resulting silver-free filtrate was termed "Silver Filtrate (IIB)."

When added to the basal medium, IIA alone was approximately 2 to 6 per cent as active as the starting material, while IIB was 12 to 25 per cent as active. Recombination of the two factors produced from two to four times the effect which would be expected from a simple summation of effects of the two factors. The authors quote G. W. Kidder and V. C. Dewey (*Arch. Biochem., in press*) as stating that Factor IIB can be replaced by copper ion and pyridoxal.

Factor IIA could be assayed for by utilizing the basal medium to which had been added active preparations of Factor IIB. Chromatographic adsorption studies indicated that Factor IIA consists of two chemically different forms with identical biologic activity for the protozoon. This factor was found to be relatively stabile to hydrolysis by less concentrated alkali or acid, partially precipitated by lead acetate, precipitated by saturated ammonium sulfate solution, and extractable at pH 3.0 by butanol, but not by amyl acetate or chloroform. It was demonstrated to be different from pimelic acid, 2-methyl-1,4-naphthoquinone, ascorbic acid, thioglycollic acid, vaccenic acid, oleic acid, Tween 80, creatine, creatinine, and indoleacetic acid, as well as distinct from a strepogenin concentrate, animal protein factor, and cow manure factor. Also, Factor IIA concentrates were found to be inactive for Lactobacillus lactis Dorner. The authors suggest the term "protogen" for Factor IIA which is necessary for growth of this protozoon.

Relatively high concentrations of protogen were found in alfalfa meal, cerophyll, Difco yeast extract, and autolyzed pork pancreas. Tomato juice, molasses, yellow corn, and buttermilk were poor sources of the factor.

The role of these nutritional substances in the economy of other organisms is unknown. The present study again illustrates the value of employing a number of test organisms in exploration of nutritional requirements.

VITAMIN D INTOXICATION

Two additional cases of intoxication resulting apparently from the deleterious effects of large doses of vitamin D have been described by C. K. Donegan, A. L. Messer, and E. S. Orgain (*Ann. Int. Med.* **30,** *429 (1949)*).

T. S. Danowski, A. W. Winkler, and J. P. Peters (*Ann. Int. Med.* **23,** *22 (1945)*) several years ago described 2 patients suffering from vitamin D intoxication, one of whom was taking vitamin D produced by the electrical activation of heat-vaporized ergosterol (Ertron). In both instances there was evidence of decreased renal function and one patient exhibited soft tissue calcification. Subsequently, S. Freeman, P. S. Rhoads, and L. B. Yeager (*J. Am. Med. Assn.* **130,** *197 (1946)*) described lassitude, weakness, anorexia, skin eruption, involvement of the conjunctivae, renal insufficiency, anemia, and eosinophilia in 2 subjects taking Ertron. The postmortem findings in 5 cases of hypervitaminosis D with metastatic calcification were described by J. M. Bauer and R. H. Freyberg (*Ibid.* **130,** *1208 (1946)*). The pathologic changes consisted chiefly of calcification involving the arterial vascular system and the kidneys. In four instances metastatic calcification was considered to be the cause of death. Still another death associated with Ertron therapy was described by P. Kaufman, R. D. Beck, and R. D. Wiseman (*Ibid.* **134,** *688 (1947)*) whose patient developed massive metastatic tissue calcification, renal insufficiency, and anemia.

The first of the 2 patients described by Donegan *et al.* (*loc. cit.*) had had rheumatoid arthritis and had been taking Ertron for a prolonged period of time in doses as high as 150,000 U.S.P. units of vitamin D per day. Weakness, lassitude, metastatic calcification, disturbance of vision, lesions of the conjunctivae, and renal insufficiency developed. Following discontinuance of Ertron therapy and the institution of a low calcium regimen, the serum calcium gradually decreased from 13.4 mg. per cent to normal levels. There was, at the same time, some amelioration of the symptoms but no significant improvement in renal function was observed in eleven months. The conclusion was reached that irreversible damage had been produced in the kidneys.

In the second case there was low back pain, severe renal impairment, elevated serum calcium levels (15 mg. per cent), and marked anemia. During the ensuing six months, after ingestion of Ertron was stopped, there was marked improvement of renal function, blood calcium fell to a normal level, and the anemia disappeared.

The exact mode of action of vitamin D in hypervitaminosis D has not been explained. The effect is not simply due to an increased absorption of calcium. E. Watchorn (*Biochem. J.* **24,** *631 (1930)*) showed that, regardless of the increased absorption, the hypercalcemia and tissue calcification are not due entirely, if at all, to increased absorption. The amounts retained in the body are actually less than normal. A. T. Shohl ("The Vitamins," *Chapter 24, p. 459, Am. Med. Assn., Chicago (1939)*) stated that when the dose of vitamin D is one thousand times the therapeutic dose, the deposition of minerals at the epiphysis is made at the expense of the shaft. The balances of calcium and phosphorus then become negative, and metastatic calcification takes place. In experimental animals, there is weight loss, anorexia, and diarrhea and death may occur in five to fourteen days.

Donegan and associates offer the opinion that intoxication from administration of massive doses of vitamin D may be more widespread than reports in the literature indicate, for the clinical picture may masquerade as renal disease or even hyperparathyroidism. They point out that in all cases of hypercalcemia, hypervitaminosis D should be considered in the differential

diagnosis. One possible differentiating feature is the greatly increased serum alkaline phosphatase observed in hyperparathyroidism as compared with the essentially normal values which have been found in cases of Ertron poisoning.

ASSAY AND DISTRIBUTION OF VITAMIN B_{12}

The isolation of vitamin B_{12} as a growth factor for Lactobacillus lactis, and the subsequent demonstration of its great activity in control of pernicious anemia in man, in promotion of the growth of rats and chicks on rations deficient in "animal protein factor," or on rations inducing hyperthyroidism, demonstrate the metabolic importance of this substance for a variety of forms of life. For this reason, reliable methods for its determination become of considerable importance.

Only a beginning has been made in the solution of this problem. Although a variety of lactic acid bacteria requires the growth factor, their response to it is not specific, since reducing agents such as ascorbic acid, and degradation products of animal nucleic acid (desoxyribosides of thymine, hypoxanthine, adenine, cystosine, and guanine) all give similar growth responses, although at much higher concentrations (E. Kitay, W. S. McNutt, and E. E. Snell, *J. Biol. Chem.* **177,** *993* (*1949*)). Although these organisms are useful in determination of vitamin B_{12} in rich sources of this vitamin, they cannot be relied upon at present to give meaningful values for many crude materials which are low in the vitamin.

Discovery of a free-living protozoan which requires this vitamin (S. H. Hutner and coworkers, *Proc. Soc. Exp. Biol. Med.* **70,** *118* (*1949*)) may therefore prove of considerable value. This organism, Euglena gracilis var. bacillaris, grows well in the presence of light and in a simple medium consisting chiefly of inorganic salts, potassium citrate, sodium butyrate, and monosodium glutamate, provided two vitamins, thiamine and the crystalline antipernicious anemia factor (APA, vitamin B_{12}) are present. Only about 0.01 millimicrogram of the crystalline APA factor per milliliter of medium is required to permit one-half maximum growth of this test organism. In contrast to lactic acid bacteria so far tested, this organism does not grow with thymidine in place of the crystalline APA factor. This apparent specificity of the test organism should be further tested, and may then provide a specific method for determination of the vitamin in natural materials. Such application of the organism has not been reported as yet.

In a preliminary attempt at an appraisal of the vitamin B_{12} content of meats, U. D. Register, U. J. Lewis, H. T. Thompson, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **70,** *167* (*1949*)) have employed a short-term assay with rats. The animals were depleted for two weeks on a corn-soybean ration containing added vitamins, salts, and 0.06 per cent of iodinated casein (Register, W. R. Ruegamer, and Elvehjem, *J. Biol. Chem.* **177,** *129* (*1949*)), then fed graded amounts of the substance to be tested during a second two week period. Reticulogen or crystalline vitamin B_{12} are highly effective in promoting growth on this ration, 0.2 microgram of vitamin B_{12} per day increasing the average weight gain over the two week period from about 36 g. (average for the control group) to 90 g. Assay of two samples of beef (round steak) showed a concentration of 2 micrograms of vitamin B_{12} per 100 g. of sample. One sample of pork (shoulder) from a sow which had showed normal lactation contained 1 microgram of vitamin B_{12} per 100 g., a second sample from a sow showing abnormal lactation (as evidenced by early death of the young) con-

tained much less than this amount. A sample of reticulogen, in contrast, contained 2.0 mg. of vitamin B_{12} per 100 g. by this assay, or about 1 microgram per U.S.P. unit.

The authors suggest that these variations in the vitamin B_{12} content of pork may explain the wide variability in the ability of pork muscle, fed as the protein source, to produce normal lactation in female rats, as opposed to the constantly good lactation performance noted when beef muscle was the protein source (L. M. Henderson, B. S. Schweigert, A. K. Mozingo, and C. A. Elvehjem, *J. Nutrition* **36,** *479* (*1948*)).

The specificity of this assay method for vitamin B_{12}, like that of the protozoal method, has still not been adequately tested. These preliminary results are suggestive, however, of the nutritional problems which may be clarified once reliable figures for the vitamin B_{12} content of feed and foodstuffs become available.

DIET AND EPIDEMIC THYROTOXICOSIS IN DENMARK

The relation of diet to thyrotoxicosis as well as to other forms of thyroid disease is a complicated subject and has been the object of many investigations. The interrelation between thyroid function and vitamin metabolism was reviewed previously (*Nutrition Reviews* **2,** *87* (*1944*)).

During the German occupation of Denmark a marked rise in frequency of thyrotoxicosis was observed and was commented on by E. Meulengracht (*Acta med. Scandinav.* **121,** *446* (*1945*)). An analysis of this epidemic of thyrotoxicosis in Denmark has been made by K. Iversen ("Temporary Rise in Frequency of Thyrotoxicosis in Denmark 1941–1945," *Rosenkilde & Bagger, Copenhagen* (*1948*)). Since occupying armies generally disrupt food supplies, this factor was investigated thoroughly by Iversen as a possible cause of the sudden rise in the incidence of thyrotoxicosis. In addition, other factors possibly influencing morbidity from thyrotoxicosis were studied, such as psychic trauma, infectious diseases, alterations in intake of iodine and of thyroid medication, and alterations in climatic conditions.

Prior to the German occupation there had been a gradual increase in incidence of hospitalized cases of thyrotoxicosis in Denmark, that is, in 1931 there were approximately 600 cases hospitalized, while in 1940 there were almost 1400. This increase was gradual and stepwise year by year. In 1941 there were 2000 patients with thyrotoxicosis admitted to hospitals, in 1942 there were almost 3500, while in 1943 there were nearly 5000 cases admitted to hospitals. This year represented the peak, and thereafter the incidence fell off, in 1944 approximately 4500 being admitted. The increased incidence was clearly shown not to be due to increased population nor a tendency for hospitalization nor improved ability in diagnosis. The increased frequency was felt by Iversen to represent the true increase in morbidity of the disease. The possibility that psychic trauma caused by the disturbed living conditions during the German occupation might have been an important factor in the epidemic is discussed at length.

The author investigated the reports from World War I of the incidence of thyrotoxicosis. It was believed at that time in a number of incidences that a rise in thyrotoxicosis had occurred, nevertheless careful investigation of these reports by Iversen failed to substantiate them in most instances. In fact, the most reliable statistics from World War I showed that thyrotoxicosis actually decreased rather than increased and that there was no evidence to suggest that "war Basedow" actually existed as a clinical or epidemiologic phenomenon. Furthermore, the Denmark epidemic was apparently unique during World War II. From both published reports and personal communications, Iver-

sen demonstrated that in other occupied countries as well as countries threatened with occupation and repeatedly bombed, as in Britain, thyrotoxicosis did not significantly increase or actually decreased. In addition, the psychic strain from German occupation of Denmark appeared to be most severe during the years 1944 to 1945, whereas the marked rise in incidence of thyrotoxicosis had occurred before this time. It was concluded that psychic trauma did not influence the incidence of thyrotoxicosis in Denmark.

In Iversen's study there appeared to be no etiologic connection between infectious diseases, especially rheumatic fever, and the incidence of thyrotoxicosis. Considering the possibility that alterations in the intake of iodine might have influenced the morbidity due to thyrotoxicosis the author reviewed the literature and came to the conclusion that if there were a condition "iodothyrotoxicosis" its basis was a nontoxic nodular goiter, which is almost unknown in Denmark. Thus, iodine ingestion would appear to have had no etiologic connection with the epidemic. Likewise, medication with thyroid could not have been considered important etiologically since only a small percentage of the patients with thyrotoxicosis had a history of preceding thyroid medication.

The relation between climatic changes and the incidence of thyrotoxicosis is also discussed. In Denmark just before and simultaneous with the rise in the incidence of thyrotoxicosis there were three unusually cold winters in succession, Iversen reports. It was thought that this change in climate might have had an important influence on the incidence of the disease, although the same cold winters were found in Holland and Belgium where the incidence of thyrotoxicosis decreased. However, much less food was available in Holland and Belgium than in Denmark during these years, which could have a depressing effect upon the incidence of the disease.

The effect of changes in diet upon the rise in frequency of thyrotoxicosis in Denmark is considered in some detail by Iversen. It was concluded that the temporary rise in the frequency of the disease was to some extent at least connected with the extraordinary dietary conditions during the war, although the evidence for this was by no matter of means conclusive. It is well known that starvation will reduce the basal metabolic rate, although it has been concluded by J. Beattie and P. H. Herbert (*Brit. J. Nutrition* **1,** *185 (1948)*) that although the metabolic rate per square meter of body surface was reduced in starvation, the metabolic activity of the tissues was not significantly different from the normal. As Iversen points out, however, there was a decrease in thyrotoxicosis in both Finland and Germany during World War I.

Similar studies in Belgium and in malnourished patients in the United States indicate that loss of weight reduces the incidence of thyrotoxicosis. In addition to considering the quantity of food available, Iversen discusses several of the nutrients which may have a specific thyrotoxic or antithyroid activity. With regard to the latter, the presence of antithyroid substances in food is extensively discussed, as well as the use of synthetic antithyroid substances in the treatment of thyrotoxicosis.

The Danish diet during the war was carefully analyzed by Iversen. In general, during the German occupation, the Danish diet appeared to have been adequate; in fact with regard to protein and certain protective foods there was an actual increase of consumption during the war, while there was some decrease in consumption of fat and carbohydrate. Following 1941, the import of vegetable oil almost completely stopped (coconut oil, sesame oil, and soybean oil). Whether the reduction in these oils in the food consumed by the Danes during the war had any influence on the incidence of thyrotoxicosis can only be supposed. Nevertheless, as Iversen points out, soybean oil or at

least soybean cakes contain antithyroid substances or activity (R. McCarrison, *Indian J. Med. Res.* **21,** *179 (1933)*).

It was also considered possible, although unproved by experiment, that antithyroid substances present in soybean meal fed to cows might be present in the milk and exert its activity in this way. Using the diminished quantity of soybean oil as a possible explanation for the increased incidence of thyrotoxicosis presents one difficulty, as Iversen notes. That is, that the importation of soybean oil began to fall off in 1938 and 1939 and actually none was imported from 1941 on; yet the rise in incidence of thyrotoxicosis, although beginning at approximately the same time as the fall in imports of soybean oil, did not continue as long, actually falling off beginning in 1944. It is, of course, true also that the importation of soybean oil and cake fell off in other northern European countries during the war, but here again these countries suffered from markedly decreased food supplies, which was not the case in Denmark.

Since cabbage and similar materials also contain antithyroid substances, Iversen investigated the ingestion of these substances before and during the war. Reliable information was difficult to obtain but an approximate estimate was presented. No particular changes in consumption were observed so that the influence of antithyroid materials contained in these vegetables could not be said to influence the morbidity rate from thyrotoxicosis in Denmark.

It was also pointed out by Iversen that protein contained some activity in increasing at least the size of the thyroid in animals. Dietary protein increased somewhat during the war. Whether this had a similar influence to the elimination of soybean oil could of course not be proved, but is suggested. Iversen concluded, as noted above, that changes in the diet, particularly the absence of soybean oil, might have led to the increase in thyrotoxicosis observed in Denmark during the war and that the colder climate mentioned above might have had an influence as well.

E. Meulengracht (*Arch. Int. Med.* **83,** *119 (1949)*) has proposed a different mechanism to account for the Danish thyrotoxicosis epidemic. As evidence against the disappearance of the antithyroid activity of soybean oil, Meulengracht cites the decline in incidence of thyrotoxicosis before the importation of soy began again and the failure of the epidemic to occur in Sweden although soybean oil was apparently as severely limited in that country as in Denmark.

As an alternative, Meulengracht suggests that a specific infective agent might be responsible. To the author the epidemiology of the epidemic is better fitted to this cause than to any other. Although this possibility must, of course, be considered, there is little evidence that thyrotoxicosis is ever related to an infectious agent, as the author himself points out. It seems unlikely that a final answer to the etiology of this epidemic will be forthcoming, but the possibility of a nutritive factor should spur research in this field.

SPRUE IN MILITARY PERSONNEL

Of interest is the summary report of three outbreaks of sprue during 1943–1944 among military personnel in Burma (F. Ayrey, *Tr. Roy. Soc. Trop. Med. Hyg.* **41,** *377 (1947)*). The report is based on approximately 3000 cases diagnosed as mild, moderate, or severe sprue which occurred among British and Indian troops stationed in three separate areas. The outbreaks were epidemic in nature and were similarly manifested in the British and Indian groups.

The clinical features included early signs or symptoms of weakness, anorexia, and flatulence, followed by diarrhea (especially

in the early morning), sore tongue (glossitis), loss of weight, muscular cramps, anemia (usually macrocytic), flat oral glucose tolerance curves, and slight to moderate steatorrhea. A high percentage of the cases occurred in relation to malarial infestation or dysentery. Considerable variability existed in the findings from case to case, but the total experience was consistent with the author's thesis that the syndrome encountered was "tropical sprue in various stages of development."

Ayrey notes that the clinical picture exhibited by the milder cases occurring in these epidemics conformed to the syndrome which others have termed para-sprue (*Nutrition Reviews* **3**, *181* (*1945*))—glossitis, anemia, and diarrhea without pronounced steatorrhea or extreme emaciation. Simultaneously, within the same units untreated or partially treated patients often developed the full blown picture of classic sprue. The author contends that "para-sprue" is but an early or mild form of sprue and, therefore, should not be separated diagnostically. He states, "Dissimilar in appearance as these earlier and later phases may be, they nevertheless should be regarded as stages in development of tropical sprue." If this viewpoint is correct it may be that a similar relation exists between sprue and nutritional macrocytic anemia encountered in the southern United States. Such a relation has been postulated (W. J. Darby, *Vitamins and Hormones* **5**, *119* (*1947*)).

The first outbreak which Ayrey describes appeared in British troops who had been stationed in Indian from ten to sixteen months. The second epidemic affected both British and Indian personnel (the latter more severely). The third attacked an Indian artillery regiment which had been surrounded by the Japanese and subjected to six months of "most arduous conditions." In all cases, the actual dietary available to the troops had been very low in eggs, milk, meat, and vegetables. The first outbreak had been preceded by a diet high in fatty foods and tinned products, many of the latter having been unsatisfactory. These canned foods, especially the fats, were described as "rancid" or "unwholesome" in taste, the expiration date for consumption had frequently passed. The tins were often "blown" with bulging lids. It is apparent that the tropical storage conditions may have altered the nutritive quality of these foods or at least have made them unpalatable. Accordingly, there seems a reasonable basis for Ayrey's argument that these troops had lived on a diet low in B-complex vitamins and possibly nutritionally inferior in other respects for a considerable period prior to the appearance of the epidemics. The investigator interprets his observations as supporting the view that "tropical sprue rightly belongs to the diseases of malnutrition . . ."

Other reports exist of outbreaks of sprue among military personnel in India during the last war. For example, K. D. Keele and J. P. Bound (*Brit. Med. J.* **1**, *77* (*1946*)) described the general features observed in 600 cases. Noteworthy was the acute nature of the syndrome in contrast to the prolonged history of chronic diarrhea so frequently encountered in nontropical sprue (idiopathic steatorrhea). The first attack was usually of but a few week's duration when the patient reported ill, and some recovery had often set in. A relapse appeared, however, if the patient was soon returned to duty. In more severe cases the disease progressed rapidly and more vigorous and prolonged therapy was required to bring about a remission. The time of onset of glossitis and diarrhea corresponded closely, capricious appetite was characteristic of the disease, dyspepsia, heartburn, and distention were common, and vomiting was seen occasionally. Most patients lost some 25 per cent of their usual body weight. Dryness, roughness, and pigmentation of the skin were seen in some; leg cramps were not infrequent, but definite tetany was observed in only one case. Hypotension was infrequent (5 to 10 per cent). Where this finding occurred, there

existed a salt deficiency as indicated by low serum sodium and serum chloride levels (D. A. K. Black, *Lancet* **II,** *671 (1946)*). The absence of increased serum potassium in these subjects speaks against adrenal insufficiency.

In both studies a seasonal incidence in the disease was noted—the peak occurred in May and June with the lowest incidence occurring in December and January. Studies of the geographic distribution indicated that sprue was regional (Keele, *loc. cit.*). Although cases of postdysenteric sprue were encountered by Keele, the maximum incidence of sprue definitely precedes the seasonal peak for dysentery. Finally, it was noted that the syndrome developed in active troops with dietary supplies that were "balanced and adequate on paper" but which were distasteful and monotonous.

These reports leave little doubt that sprue can be an epidemic disease of military forces. They emphasize that the disorder may occur despite available supplies of supposedly adequate foodstuffs when the available dietary is distasteful and not consumed or when it has deteriorated due to poor storage conditions. Further, they again point to some relation between dysentery, malaria, and the development of the sprue syndrome.

There have also appeared follow-up observations on personnel who contracted sprue and, after experiencing various degrees of recovery, returned home. R. Drew, K. Dixon, and E. Samuel (*Lancet* **I,** *129 (1947)*) reported on the residual defects found in 26 such patients. Thirteen of these 26 showed no radiologically demonstrable abnormality of the gastrointestinal tract, while the remaining group of 13 presented changes in the bowel pattern varying in degree from mild to severe. Two of 14 of the group studied still exhibited biochemical evidences of defective fat absorption. Thus, it is obvious that the epidemic sprue that has been observed to develop in military personnel may, in some cases, give rise to slowly reversible (or irreversible) changes within the gastrointestinal tract. Presumably, recurrent relapses in such individuals establish the connection between the acute epidemic variety of the disease and the chronic, recurrent sprue more frequently seen in temperate regions.

METABOLISM OF VITAMIN B_6 IN MAN

Early studies of J. V. Scudi and co-workers (Scudi, H. F. Koones, and J. C. Keresztesy, *Proc. Soc. Exp. Biol. Med.* **43,** *118 (1940)*; Scudi, K. Unna, and W. Antopol, *J. Biol. Chem.* **135,** *371 (1940)*; Scudi, R. P. Buhs, and D. B. Hood, *Ibid.* **142,** *323 (1942)*) established that pyridoxine was rapidly absorbed from the digestive tract of man, dog, and rat; that although excretion was rapid, only 10 to 20 per cent of the test dose was recovered in human subjects; and that a portion of the pyridoxine ingested was converted to an unidentified metabolite which, like pyridoxine itself, gave a blue color with 2,6-dichloroquinone-chlororimide, and that both this product and pyridoxine were excreted partially as conjugates which could be hydrolyzed with acid. Subsequently, J. W. Huff and W. A. Perlzweig (*Ibid.* **155,** *345 (1944)*) discovered that a large portion of the ingested pyridoxine was excreted in the urine as 4-pyridoxic acid, a product which is without vitamin B_6 activity and which does not give a color reaction with 2,6-dichloroquinone-chlororimide.

Since that time it has been shown that vitamin B_6 as it occurs naturally is a complex, consisting of pyridoxine, pyridoxal, and pyridoxamine (*Nutrition Reviews* **4,** *163 (1946)*; **5,** *98 (1947)*). A differential method for the determination of these three forms of the vitamin has been developed and applied to several natural products (J. C. Rabinowitz and E. E. Snell, *J. Biol.*

Chem. **176,** *1157 (1948)*). These determinations showed that almost all, if not all, of the vitamin B_6 of animal tissues and many other products such as yeast, was present as pyridoxal and pyridoxamine. Pyridoxine was present to a greater extent in many higher plants, but here, too, pyridoxal and pyridoxamine comprised much of the total vitamin B_6 present. Thus in natural dietaries, it is probable that most of the vitamin B_6 is ingested as pyridoxamine and pyridoxal, rather than as pyridoxine. These compounds are known to have the same growth-promoting action as pyridoxine (*Nutrition Reviews* **5,** *98 (1947)*) in animals, and each of them is converted by animal tissues to pyridoxal phosphate (W. D. Bellamy, W. W. Umbreit, and I. C. Gunsalus, *J. Biol. Chem.* **160,** *461 (1945)*), but aside from these observations nothing is known of their metabolism by animals.

For this reason, a study (J. C. Rabinowitz and E. E. Snell, *Proc. Soc. Exp. Biol. Med.* **70,** *235 (1949)*) of the metabolic disposal of pyridoxal, pyridoxamine, and pyridoxine by adult, male, human subjects on normal diets, is of interest. Following collection of a twenty-four hour control sample of urine, a 100 mg. sample of pyridoxal hydrochloride, pyridoxamine dihydrochloride, or pyridoxine hydrochloride was administered orally to each of 3 subjects. Urine samples were collected two, five, eight, twelve, and twenty-four hours after administration of the test dose. All samples were hydrolyzed with acid to yield maximum values for vitamin B_6, then assayed microbiologically for pyridoxal, pyridoxamine, and pyridoxine, and fluorometrically for 4-pyridoxic acid. The essential findings were as follows:

Following ingestion of each form of the vitamin a rapid increase in urinary excretion of the form of the vitamin fed occurred. This reached maximum levels one to three hours after ingestion of the dose, and returned to basal levels within about eight hours. Administration of pyridoxine greatly increased the excretion not only of pyridoxine, but also of pyridoxal, pyridoxamine, and 4-pyridoxic acid. Administration of pyridoxamine increased greatly the excretion of pyridoxamine, pyridoxal, and 4-pyridoxic acid, but no pyridoxine could be detected in the urine. Similarly, pyridoxal administration greatly increased excretion of pyridoxal and 4-pyridoxic acid, slightly increased pyridoxamine excretion, but failed to cause any detectable excretion of pyridoxine.

Thus, pyridoxine appears to be converted in significant proportions to pyridoxal and pyridoxamine within the human body, but no evidence that either of the latter products gives rise to pyridoxine could be found. The common form of the vitamin which appears in the urine following administration of any one of the three compounds is pyridoxal. All three of the forms of vitamin B_6 are oxidized within the body to 4-pyridoxic acid. The amount of the latter compound formed from pyridoxal, however, was significantly greater than that formed from either pyridoxine or pyridoxamine. Because of this, and since 4-pyridoxic acid was quantitatively the most prominent of all excretion products, the recovery of the test dose of pyridoxal was respectively, 70 and 64 per cent of that fed for each of 2 patients. This total recovery was much higher than that obtained with pyridoxine (45 and 38 per cent) or with pyridoxamine (31 and 31 per cent). Nothing is known of the fate of that portion of the test dose not recovered in the urine. The rapid return of excretion levels to normal argues against tissue storage, and comparison with previously published data indicates that complete absorption of the test dose probably occurred. It is postulated that a portion of the vitamin is converted to products still unknown, or is completely oxidized within the body.

Whatever its fate, the data indicate that the three forms of vitamin B_6 are metabolized in a similar fashion. Each gives rise to pyridoxal, which this and other evidence would indicate to be the form of the vitamin

actually used for metabolic purposes (i.e., conversion to pyridoxal phosphate, the catalytically active coenzyme form of vitamin B_6). A major portion of each form is eventually oxidized and excreted as 4-pyridoxic acid.

The study does nothing to clarify the puzzling observations (*Nutrition Reviews* **5**, *98* (*1947*)) that pyridoxal and pyridoxamine are less active than pyridoxine in promoting growth of vitamin B_6 deficient animals when fed with the ration, but are equally active when administered by injection or fed separately apart from other food.

INCORPORATION OF RADIOACTIVE COMPOUNDS INTO NORMAL AND TUMOR TISSUE

Radioactive isotopes have frequently been considered as possible materials to be used for the therapeutic irradiation of deeply located neoplasms and metastases. The hope of success in a treatment of this type must be based on the logistics that the particular compound carrying the radioactive isotope would be localized in the malignant tissues in such a high concentration that the emanations from the radioisotope would be damaging to the neoplasm. However, the radioactive compounds must not be collected in sufficient amounts in the surrounding normal tissues as to be toxic to them. At the present time the radioactive tracers are of far greater fundamental importance for the study of the metabolic processes in malignant growths.

J. C. Reid and H. B. Jones (*J. Biol. Chem.* **174**, *427* (*1948*)) determined the amounts of radioactivity in the various tissues of mice after the administration of relatively high amounts of DL-tyrosine labeled with radioactive carbon in the beta position. This was one phase of a general project to study the administration of various melanin precursors labeled with radioactive carbon to mice with melanosarcoma. This investigation seemed to have considerable promise by reason of the rapidity of melanin formation in the cells of the melanosarcoma. The administration of a labeled precursor of melanin would be expected to result in an especially high radioactivity in the melanin within the malignant cells.

The mice used in this experiment were 3 adult males of the dba strain which had been inoculated bilaterally with melanosarcoma S91. After the tumors had been allowed to develop for five weeks, each mouse was given a single injection of labeled DL-tyrosine in physiologic saline via the tail vein. After injection the mice were placed together in a glass cage with provision for collecting their excreta and for absorbing all radioactive and normal carbon dioxide exhaled in sodium hydroxide solution.

At the end of twelve hours, one mouse was removed from the cage and killed. The second and third ones were killed at twenty-four and seventy-two hours, respectively. The tissues of each mouse were dissected immediately after death in as quantitative a procedure as possible. The tissues were weighed and representative aliquots saved for evaluation of their radioactivity. In this way, the total radioactivity in each tissue could be calculated. This made it possible to calculate a balance between the total amount of radioactivity administered and the amount found in the various tissues, the expired carbon dioxide, and the excreta. The fresh tissues were dried immediately in a vacuum oven at 60°C. The water resulting from these drying processes was collected and examined for radioactivity to determine whether any of these radioactive compounds in the tissues were volatile.

Less than 0.01 per cent of the administered dose was found to be lost through volatilization. Evaluation of the radioactivity of the specimens involved the burn-

ing of an aliquot in an analytical-type combustion train to carbon dioxide; the resulting gas was absorbed in sodium hydroxide solution. Barium carbonate was precipitated from the latter solution and the specific radioactivity of the barium carbonate determined. Where the tissues were too small to handle conveniently, such as the pylorus, adrenal glands, and gall bladders, benzoic acid was added to the sample in the combustion boat as a carrier. The dilution introduced by this procedure was believed to be immaterial in the determination of the specific radioactivity of the tissue (the total amount of radioactivity in the precipitated barium carbonate divided by the weight of tissue burned). Unfortunately the total radioactivity of these samples was on the lower edge of the useful range.

The specific radioactivity expressed in counts per minute per milligram of dry tissue indicated that there had not been a strikingly high incorporation of radioactive materials into the tumors despite the rapid rate of melanin formation. In the mouse which was killed at the end of twelve hours after injection, the specific activity was found to be 24.4 counts per minute per milligram of tumor tissue, with lesser amounts in the mice that were killed at later intervals.

The adrenals, intestines, kidneys, plasma, and thyroids were all found to have higher specific activities than the tumor tissue. No part of the body tested was found to be free of radioactivity. The total amount of radioactivity still in the tissues of the mouse at the end of twelve hours was about 60,000 counts per minute, i.e., about 20 per cent of the administered dose. Of this amount, 9400 counts per minute were present in the melanosarcoma, 11,500 in the intestines, 7000 in the liver, 8800 in the muscle, 3900 in the bone, 4900 in the skin and hair, and 1990 in the kidneys. The other organs had relatively small amounts in comparison.

In an *in vitro* experiment using the liver-slice technic, P. C. Zamecnik, I. D. Frantz, R. B. Loftfield, and M. L. Stephenson (*J. Biol. Chem.* **175,** *299 (1948)*) determined the rate of incorporation of DL-alanine labeled in the carboxyl position with radioactive carbon into the proteins of normal and malignant rat livers. Young male rats were fed a diet containing para-dimethylaminoazobenzene for two months and then a normal diet for an additional two months. At the end of the second period, exploratory laparotomies were performed; all rats with small isolated hepatomas were noted. After another month, animals known to have hepatomas were killed by decapitation and the livers removed. For most experiments, hepatomas which were about 1 cm. in diameter and had no evidence of necrosis were dissected. In other experiments, the periphery of larger tumors with necrotic areas was used.

Areas of the liver which were not grossly malignant were selected for preparation of slices. In addition, livers from rats which had not been fed para-dimethylaminoazobenzene were prepared for study. Slices of all samples were made and then incubated at 37°C. for three and a half hours in Krebs-Ringer phosphate solution containing labeled DL-alanine, under an atmosphere of oxygen. After incubation the proteins were precipitated and hydrolyzed. The ninhydrin procedure was used to split off the alpha-carboxyl groups in the amino acids and the gamma-carboxyl group of aspartic acid. The carbon dioxide released thereby was trapped with barium hydroxide to form barium carbonate which was used for determination of the radioactivity.

In twenty runs the average radioactivity detected in the barium carbonate from the proteins in a liver slice from a normal rat was 37 counts per minute. In contrast, sixteen tests with hepatoma slices gave an average value of 255 counts per minute. The average value for fourteen experiments with slices from relatively normal areas of hepatoma-bearing livers was 91 counts per

minute, which represents an appreciable increase over the liver from normal rats. A preponderance of the activity was accounted for in the alanine fraction of the proteins. Glycine labeled with radioactive carbon in the carboxyl position was also added to a series of incubation flasks under similar conditions. The slices of hepatomas had incorporated radioactive glycine into their proteins at a much higher level than normal regions of the same livers which had a higher incorporation rate than normal livers.

These data indicate clearly the greater rate of synthesis of new proteins which was occurring *in vitro* in the slices from the malignant growths initiated by the effects of para-dimethylaminoazobenzene. The average amount of barium carbonate obtained from the hepatoma slices in the alanine experiments was less than from other areas of the same livers which in turn yielded less barium carbonate than slices from normal livers. This was an indication that there was a higher amount of protein present in the normal liver slices than in the hepatomas.

I. D. Frantz, P. C. Zamecnik, J. W. Reese, and M. L. Stephenson (*J. Biol. Chem.* **174,** *773 (1948)*) studied the effect of dinitrophenol on the incorporation of DL-alanine into normal and malignant liver slices. Addition of dinitrophenol at low levels to the above system inhibited the incorporation of alanine into protein in normal and tumor tissue but permitted oxygen consumption to continue. In higher concentrations, dinitrophenol had an inhibitory effect on respiration in the hepatoma slices but essentially none in the normal liver slices. The latter observation might suggest that there was a somewhat different respiration pathway in the tumor tissue.

The several studies described above indicate the value of radioactive tracers in the comparative evaluation of the metabolic processes in normal and tumor tissues. Through continued investigations of this type, there is real hope that the specific metabolic disturbances which occur in tumor tissue will be understood and ways devised to inhibit these metabolic pathways without injurious effects upon normal metabolism.

CHOLINE REQUIREMENT OF THE GERMAN COCKROACH

The importance of studying the comparative nutritional requirements of widely divergent forms of life to development of a true science of nutrition, and the advantages of including insects among the organisms investigated, have been emphasized in this journal (*Nutrition Reviews* **3,** *173 (1945)*). Previous investigations with insects have established that they require for growth many of the "B-vitamins" required in common by many microorganisms and by higher animals, and that the nutritional requirements of various species differ in detail.

An investigation by J. L. Noland and C. A. Baumann (*Proc. Soc. Exp. Biol. Med.* **70,** *198 (1949)*) illustrates the latter point. Results with previously studied insects indicated that choline was not essential for growth, its omission from the basal ration resulting at most in retarded growth. These authors found, however, that omission of choline from the diet of newly-hatched German cockroaches (Blattella germanica) resulted in complete cessation of growth within ten days, and death of all of the nymphs within thirty days. For these studies a synthetic ration containing vitamin-free casein, glucose, salts, corn oil, cholesterol, and eight synthetic "B-vitamins" was employed. The major difference between such a diet and that used in animal studies lies in the omission of fat soluble vitamins (which have so far not been

implicated in insect nutrition) and in the addition of cholesterol, which is required preformed by many insects.

As indicated previously, roaches failed to grow or survive on this ration when choline was omitted. At a level of 500 micrograms of choline per gram, the growth rate was very slow and only one fifth of the insects reached maturity. With 1000 micrograms per gram, the insects reached maturity, but growth was suboptimal. Growth was good, however, at 2000 and 4000 micrograms of choline per gram of ration; a larger amount, 8000 micrograms per gram, was somewhat toxic. This requirement for optimum growth compares rather closely with that necessary for good growth in chicks fed a minimum level of methionine, which is reported to be 2000 micrograms per gram of ration (D. S. McKittrick, *Arch. Biochem.* **18,** *437 (1948)*).

The specificity of the choline requirement was investigated by attempting to substitute related compounds for choline in the diet. Methionine, diethylaminoethanol, and ethanolamine were completely ineffective as substitutes for choline. Betaine, however, promoted growth which was fully equal to that supported by equal levels of choline. When the same four compounds were tested in the presence of a suboptimal level of choline (500 micrograms per gram of ration) results with betaine and aminoethanol were unchanged, but methionine and dimethylaminoethanol increased growth, survival, and maturation, although not to the same extent as equimolar levels of choline.

The choline content of roaches grown on the various diets was measured by means of an assay employing the choline deficient mutant of Neurospora crassa (N. H. Horowitz and G. W. Beadle, *J. Biol. Chem.* **150,** *325 (1943)*). Newly-hatched nymphs contained about 1080 micrograms per gram of live weight; adults maturing on rations supplying 1000, 2000, and 4000 micrograms of choline per gram contained an average of 1620, 1730, and 2780 micrograms of choline per gram of live weight, respectively. Roaches grown with betaine in place of choline contained choline in amounts approaching those found in insects grown with similar levels of choline. Thus the betaine (which is without activity for the assay organism) had been transformed to choline during growth either by the roach itself, or by associated microorganisms.

These results in the roach differ in several respects from those in higher animals. In the chick, betaine only partially replaces the choline requirement, whereas in the insect, betaine is a complete supplement. Dimethylaminoethanol plus methionine supports fair growth in the chick in the absence of choline; this is not true in the insect. Similarly, the rat can utilize ethanolamine plus methionine in place of choline; the cockroach does not.

These differences emphasize that in animals, as in microorganisms, the sequence of synthetic reactions leading to formation of a given compound such as choline may be deficient at any one of several stages, that the deficient reactions vary from one organism to another, and that these different synthetic capacities give rise to significantly different behavior toward compounds related in structure to choline. It is only by study of these relations in a variety of species that the various possible interrelations become clear. Which of these, if any, will ultimately prove to hold for the human species is a matter for future research.

THIAMINE SUPPLEMENTATION AND LEARNING CAPACITY

R. F. Harrell began studies on the effects of thiamine supplementation on learning in 1941 ("Effect of Added Thiamine on Learning," *Teachers College, Columbia University, New York (1943)*). The initial study extended over six weeks and children in a

Virginia orphanage served as subjects. The diet, of unspecified caloric content, was estimated to provide 0.9 mg. of thiamine per child daily. The experimental subjects received daily a supplement of 2 mg. of thiamine in the form of a tablet, while the control children received placebos. All children were given a series of practice sessions on eighteen tasks including mental functions, motor skills, and strength.

The supplemented group showed larger practice gains than the control group. The difference was noted already on the second trial, i.e., within one week on the dietary regimen. The supplemented subjects continued to exhibit larger gains throughout the six week period. The total mean gains, expressed as percentage of the initial values, were 171.5 and 183.5, respectively. The difference between the gains of the basal and the supplemented subjects was statistically highly significant for two tests and significant for five tests. The finding that addition of thiamine to a regular diet appeared to improve the learning capacity was reported to be striking. The need for further work along these lines was obvious.

Dr. Harrell resumed the study in September 1942. The experimental group was maintained on 2 mg. of thiamine supplement for a year. Again, the experimental subjects surpassed the control group in all activities which were measured. In eight out of fifteen tests the difference between the experimental and the control subjects reached a level of statistical significance. From September 1943 on, some subjects were continued on the same dietary regimen while for others the supplement-placebo status was reversed. Among the subjects whose dietary status was unchanged, the supplemented subjects retained or increased their superiority. The results of the dietary reversal were not uniform. The results reported in preliminary publications have been reviewed (*Nutrition Reviews* **4,** *343* (*1946*)). A full report on the experiment has been published by Harrell ("Further Effects of Added Thiamine on Learning and Other Processes," *Teachers College, Columbia University, New York* (*1947*)).

The study was repeated by E. C. Robertson, C. M. Tatham, N. F. Walker, and M. R. Weaver (*J. Nutrition* **34,** *691* (*1947*)). Thirty-six pairs of twins were used and a large battery of psychologic tests was applied. No significant differentiation between the supplemented and unsupplemented subjects was obtained (*Nutrition Reviews* **6,** *174* (*1948*)).

Negative evidence was obtained also by H. Guetzkow and J. Brozek (*Am. J. Psychol.* **59,** *358* (*1946*)). After twenty-one days of severe deficiency of the diet in vitamins of the B-complex (30 micrograms of thiamine, 50 micrograms of riboflavin, and 400 micrograms of niacin per day) there was no difference between the deficient and supplemented subjects in the rate of improvement in Code Learning Test. Also, there was no impairment in the rate of learning in the test of Underlining 4's. Both tests had been used in Harrell's 1941 study.

Dr. Harrell in her first report mentioned that she tested the influence of yeast supplement on the learning capacity of the white rat (*1943, p. 2*). These investigations were considered as the first precise tests of the hypothesis that "learning is so related to the nutrition of the body that an increase in learning tends to follow increase in thiamine intake" (*Ibid., p. 1*). Up to that time the idea was more a clinical hunch on Dr. Harrell's part rather than a rigorously formulated hypothesis. The results of the animal experiments were considered to support the hypothesis but have not been reported in the literature.

D. E. Lush ("The Effects of Graded Amounts of Vitamin B_1 on the Maze Learning of the White Rat," *Unpublished Ph.D. Thesis, Fordham Univ.* (*1944*)) maintained six groups of growing rats on varied thiamine intake. Over the range used in Lush's experiment (from 2 to 15 micrograms) no significant differences in maze learning could be demonstrated.

In Lush's study the absolute levels of thiamine intakes were low. In an experiment carried out by M. H. Marx (*J. Comp. Psychol.* **41,** *364 (1948)*) large supplements (100 micrograms daily) were added to a normal basic diet. It was believed that such a differential in thiamine intake should provide a clearcut test of the hypothesis that liberal thiamine allowance increases the learning capacity. The experimental procedure was described in an earlier paper dealing with the effects of supranormal intakes of glutamic acid (Marx, *Ibid.* **41,** *82 (1948)*). A multiple-T water maze with fourteen choice-points was used, the escape from water serving as the motive. It was felt that this motive was relatively constant and that it would not diminish with practice. At any rate, it was much more constant than, for example, the sex drive and less directly related to the physiologic action of thiamine than the hunger drive. The rats required no preliminary habituation to the water maze and normally swam until they reached the wire mesh ladder at the end of the maze.

All animals were allowed unlimited amounts of fresh water and food. Once a day all rats were given supplementary feeding of 1 ml. of fluid by means of a syringe and a blunted hypodermic needle, inserted through the mouth into the esophagus. Control animals were given distilled water. The daily doses received by the experimental animals contained 100 micrograms of thiamine hydrochloride. The animals were assigned into the experimental and the control group by splitting the litters at weaning (at twenty-eight days). They were placed on the experimental regimen within ten days after weaning.

Four weeks later the rats were tested on the water maze, with the water maintained at room temperature. The number of trials necessary to master the maze ("one perfect run" criterion) was recorded for each animal. In addition, the time for each practice trial and the number of errors were noted. The mean number of trials, total time, and total number of errors until mastery of the maze were 19.9 trials, 2069 seconds, and 272.8 errors, for the control group. The thiamine-supplemented subjects were slightly poorer learners, with the mean scores of 22.2 trials, 2653 seconds, and 288.1 errors. None of the differences was statistically significant.

The dietary regimen was continued for an additional two weeks after which a relearning test was run. Again, the supplemented rats gave a poorer performance. The scores for the control animals were 7.5 trials, 329 seconds, and 47.4 errors; the scores for the supplemented animals were 12.1 trials, 598 seconds, and 73.1 errors. The median values exhibited the same trend of differences.

The results are interpreted as demonstrating the lack of effectiveness of improving learning capacity by adding large amounts of thiamine to the normal stock diet of the white rats. Harrell's data which seemed to indicate a positive effect of thiamine supplementation on learning were reinterpreted. It was pointed out that in Harrell's study the food intake was controlled and that the thiamine-supplemented children may have been chronically hungrier than their matched controls, having greater appetites but no opportunity for a correspondingly greater food intake. The differences obtained by Harrell are interpreted as an artifact resulting from increased motivation rather than as a result of an increased learning ability.

The reinterpretation is based on the hypothesis that within the range of thiamine intake used in Harrell's work the added thiamine does increase appetite. There is no convincing evidence for this although the hypothesis is plausible, and at a low range of thiamine intake the relation between thiamine intake and appetite (or lack of it) has been well established. Marx wisely concludes that implications of the problems are so great as to justify additional well controlled experimental work on thiamine level of the diet and the capacity to learn.

NOTES

Nutrition and Starvation

The United States Air Force, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio has recently issued AF Technical Report No. 5740 entitled "The Physiological Requirements for Survival Rations." This is a reasonably detailed account of research done during and since World War II on survival rations. Interesting conclusions drawn from the report are the following: (1) Emergency rations are one of many integral components of aircrew survival equipment. (2) Indoctrination in the use and care of survival equipment (including clothing) is as important as their provision aboard aircraft. (3) Acceptability, storage stability, nutritional adequacy, and field utility are the major considerations in ration design and development. (4) If there is a limitation in weight and space allowances aboard aircraft for both food and water, the advantages of a survival ration must be appraised in terms of its cost to water balance. (5) There is currently no single emergency ration that can satisfactorily meet minimum survival requirements for all geographic areas. (6) Food is of secondary importance to the adequacy of clothing and sleeping bag for survival at low ambient temperatures. (7) The minimum water intake necessary to prevent dehydration during fasting is approximately 900 ml. per 70 kg. man per day. (8) A ration containing a small amount of high quality protein does not increase the solute load presented to the kidneys above that associated with fasting if the caloric intake is adequate. (9) Apparently no more than 1000 calories are necessary to maintain physical capacity. (10) The supply and physiologic requirement of water may present as much of a problem in the arctic as in the tropical environment. (11) If sufficient space is not available for its inclusion in survival kits, a ration providing approximately 2000 calories per day derived from 50 g. of high quality protein, 75 g. of fat, and the remainder from carbohydrate is recommended for use in rescue kits.

Diarrhea and Vitamin A Deficiency

The occurrence of keratinization of epithelial structures throughout the body in vitamin A deficiency is a well known pathologic change. Such metaplasia is manifested clinically by keratinization of the cornea (xerophthalmia), of mucous membranes, and of the skin (follicular hyperkeratosis) (*Nutrition Reviews* **1**, *203* (*1943*)). Metaplasia in the gastrointestinal tract is rarely commented upon. V. Ramalingaswami (*Indian J. Med. Sci.* **2**, *665* (*1948*)) has described 20 cases of vitamin A deficiency, in children, which were associated with severe diarrhea and responded promptly to administration of vitamin A concentrate.

The children belonged to the plantation-laboring class. Their diets were deficient in calories and among the individual nutrients, deficient in vitamin A, thiamine, and calcium especially. The average consumption of vitamin A was 460 I.U. daily. Forty per cent of the children attending the nutrition clinic at Coonoor in South India had either keratomalacia or Bitot's spots indicating severe vitamin A deficiency. About half of the children with these lesions also had diarrhea.

The children with diarrhea were all emaciated and had a dry, inelastic skin. Keratomalacia and follicular hyperkeratosis were common, as were Bitot's spots. The diarrhea was watery but without blood or mucus; its onset was insidious and its duration from seven days to six months. Entameba histolytica was searched for in the stools but not found, although Ascaris ova were present in 6 cases. Vincent's organisms were observed in the stools in 3 cases and were thought possibly to be pathogenic. Plasma vitamin A levels were ex-

tremely low in the 6 patients in whom the determinations were made. The highest value was 32 I.U. per 100 ml. and the lowest a serum in which no detectable Carr-Price reaction could be observed. The concentration of vitamin A in the blood in these patients is comparable to that found by others in individuals with severe vitamin A deficiency (*Nutrition Reviews* **3,** *38* (*1945*)).

All 20 patients had a dramatic control of the diarrhea by the end of forty-eight hours after administration of 1 ml. daily of a vitamin A concentrate ("Prepalin") containing 72,000 I.U. of vitamin A per milliliter. There was a concurrent improvement in the ocular symptoms when these existed. Neither kaolin nor bismuth subcarbonate nor sulfaguanidine affected the diarrhea significantly in the 5 patients in whom these preparations were tried before the vitamin A was administered. Blood levels of vitamin A after treatment are not recorded.

Deficiency States in Indian and Peruvian Children

The syndrome of malignant malnutrition, described in full by C. D. Williams (*Arch. Dis. Childhood* **8,** *423* (*1933*)) has been reviewed (*Nutrition Reviews* **6,** *270* (*1948*)). It was observed at that time that the syndrome was seen in South Africa (*Ibid.* **5,** *353* (*1947*)), tropical Africa, particularly the Uganda, and in the British West Indies. The manifestations of the syndrome vary from one part of the world to another, but it is usually characterized by a fatal issue and associated with edema, diarrhea, an enlargement of the liver, anemia, and changes in the skin resembling pellagra. In the Uganda the name Kwashiorkor has been used.

In addition to the reports already reviewed from widely separated areas two others have appeared from India and Peru. The one from South India, by V. Ramalingaswami, P. S. Menon, and P. S. Venkatachalam (*Indian Physician* **7,** *229* (*1948*)), reports 5 cases of what they call infantile pellagra in children between the ages of 6 months and 3 years.

The diets consumed by the children's parents had been observed in a previous study to be markedly deficient in calories and in many of the essential nutrients, especially protein, the vitamins of the B-complex, and vitamin A. As in other reports of the syndrome, the course was frequently fatal and was associated with the manifestations listed above. As to the skin lesions, there is considerable discussion as to whether those observed in the Uganda and in South Africa and the British West Indies are typical of pellagra. Most observers think they are not, although Ramalingaswami and co-workers believe that the skin lesions in the children they report were of a pellagrous nature. Autopsy was unfortunately refused for all 5 of these patients.

The second report, from Lima, Peru by M. Roitman ("Trastornos Cardiovasculares en los Estados Carenciales Infantiles y su Tratamiento," *Thesis* (*1946*)) describes chiefly the cardiovascular manifestations of severe malnutrition in children. Most of his patients had severe beriberi, but fatty livers were seen in a number of them. In fact the description of some of the children resembles that for malignant malnutrition seen elsewhere. Edema and frequently severe diarrhea were observed. The chief cardiovascular manifestations reported in the Peruvian article were in the electrocardiograms in which flattening and inversion of the T waves and low voltage of the QRS complex were observed in conjunction with prolonged systole and tachycardia. Roitman does not discuss extensively the other physiologic abnormalities commonly associated with beriberi.

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